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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

For: **HUMAN ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODY**

1. 39 sheets of specification.

2. 10 sheets of drawings.

3. 8 sheets of ~~Sequence~~ sequence listing.

4. Please charge the required application filing fee of **$160.00** and any other fees that may be required, to the deposit account of **Kenyon & Kenyon**, deposit account number **11-0600**. A duplicate of this sheet is enclosed.

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HUMAN ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODY

FIELD OF THE INVENTION

[01] The present invention is directed to human antibodies that are specific the epidermal growth factor receptor (EGFR). These antibodies can be used in treating neoplastic diseases and hyperproliferative disorders, among others.

BACKGROUND OF THE INVENTION

[02] Although normal cells proliferate by the highly controlled activation of growth factor receptors by their respective ligands, cancer cells also proliferate by the activation of growth factor receptors, but lose the careful control of normal proliferation. The loss of control may be caused by numerous factors, such as the overexpression of growth factors and/or receptors, and autonomous activation of biochemical pathways regulated by growth factors. Some examples of receptors involved in tumorigenesis are the receptors for epidermal growth factor (EGFR), platelet-derived growth factor (PDGFR), insulin-like growth factor (IGFR), nerve growth factor (NGFR), and fibroblast growth factor (FGF).

[03] Angiogenesis, which refers to the formation of capillaries from pre-existing vessels in the embryo and adult organism, is known to be a key element in tumor growth, survival and metastasis. Growth factors and their receptors, including epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), which activate EGFR, are thought to play a role in tumor angiogenesis. Binding of these growth factors to their cell surface receptors induces receptor activation, which initiates and modifies signal transduction pathways and leads to cell proliferation and differentiation.

[04] Members of the epidermal growth factor (EGF) receptor family are particularly important growth factor receptor tyrosine kinases associated with tumorigenesis of epidermal cells. The first member of the EGF receptor family to be discovered was EGFR, which is expressed on many types of tumor cells. EGFR has been found to be

involved in regulation of tumor cell division and growth, repair and survival, angiogenesis, invasion and tumor metastasis. Expression of EGFR is correlated with poor prognosis, decreased survival, and/or increased metastasis.

[05] EGFR is a 170 kD membrane-spanning glycoprotein with an extracellular ligand binding domain, a transmembrane region and a cytoplasmic protein tyrosine kinase domain. Binding of specific ligands (such as EGF or TNF-α) results in EGFR autophosphorylation, activation of the receptor's cytoplasmic tyrosine kinase domain and initiation of multiple signal transduction pathways that regulate tumor growth and survival. The EGFR pathway also influences production of various other angiogenic factors, such as VEGF and basis fibroblastic growth factor (bFGF), in tumors.

[06] Inhibition of EGFR on tumor cells has been shown to inhibit the growth or progression of such tumors. Various antagonists designed to block EGFR activity, including monoclonal antibodies (MAbs), are being evaluated in ongoing clinical trials evaluating the clinical utility of these antagonists for the treatment of EGFR-expressing cancers. Accordingly, MAbs that antagonize EGFR may be useful in treatment of cancer.

BRIEF SUMMARY OF THE INVENTION

[07] The present invention provides purified antibodies or fragments thereof specific for EGFR, preferably the extracellular region of EGFR, comprising anywhere from one to six complementarity determining regions (CDRs) selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13. Preferably, the antibodies of the present invention, or fragments thereof, comprise SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5. Alternatively, but also preferably, the antibodies of the present invention, or fragments thereof, comprise SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13. More preferably the antibodies of the present invention, or fragments thereof, comprise a heavy chain variable region of SEQ ID NO:7 and/or a light chain variable region of SEQ ID NO:15. Such antibodies or fragments thereof of the

present invention have various properties, including the ability to neutralize EGFR and prevent binding of a ligand of EGFR to its receptor.

[08] Additionally, the present invention provides isolated polynucleotides encoding the present antibodies or fragments thereof as well as expression vectors comprising these polynucleotide sequences operably linked to an expression sequence. Recombinant host cells comprising the expression vector, or a progeny thereof, wherein the cell expresses the present antibodies or fragments thereof are also provided. Methods are also provided for producing antibodies or fragments thereof comprising culturing these cells under conditions permitting expression of the antibodies or fragments thereof. The antibodies or fragments thereof can then be purified from the cell or medium of the cell.

[09] Also additionally, the present invention provides methods of treating tumor growth in a mammal, comprising administering to the mammal an effective amount of a present antibody. Such methods can further comprises administering to the mammal an anti-neoplastic agent or treatment. Alternatively, the present invention provides methods of treating a non-cancer hyperproliferative disease, e.g., psoriasis, in a mammal comprising administering to the mammal an effective amount of the present antibody.

BRIEF DESCRIPTION OF THE FIGURES

[10] Figure 1 shows *in-vitro* binding of IMC-C11F8 and IMC-C225 to EGFR as measured by ELISA.

[11] Figure 2 shows *in vitro* competition results of IMC-11F8 and IMC-C225 with ^{125}I-labeled EGF for EGFR binding.

[12] Figure 3 shows effects of IMC-11F8 and IMC-C225 on the phosphorylation of EGFR in BxPC3 cells. Control antibody used is IMC-1C11.

[13] Figure 4 shows inhibition of EGFR phosphorylation by IMC-11F8 and IMC-C225 in A431 cells.

[14] Figure 5 shows Western Blot analysis of EGFR phosphorylation in the presence of unstimulated control cells (lane 1), EGF (lane 2), IMC-C225 (lane 3), IMC-11F8 (lane 4) and control antibody (lane 5). Figure 5A shows phosphorylated EGFR using an anti-phosphotyrosine antibody and Figure 5B shows total EGFR in the stimulated cells.

[15] Figure 6 shows inhibition of EGF-stimulated EGFR phosphorylation by various concentrations of IMC-11F8. Figure 6A shows anti-phosphotyrosine antibody Western blot analysis of EGFR in unstimulated control cells (lane 1), stimulated cells treated with no IMC-11F8 antibody (lane 2), 15 μg/mL (lane 3), 3 μg/mL (lane 4), and 0.6 μg/mL (lane 3) IMC-11F8. Figure 6B shows total EGFR.

[16] Figure 7 shows inhibition of DiFi cell proliferation by IMC-11F8, IMC-C225 and control antibody, IMC-IC11 as assessed by an MTT assay.

[17] Figure 8 shows the specific lysis of ^{51}Cr-labeled DiFi cells treated with IMC-11F8 or IMC-C225 (ERBITUX™).

[18] Figure 9 shows the growth of A431 tumor cells in mice treated with either IMC-11F8 or IMC-C225 (CETUXIMAB). Untreated animals serve as control for tumor growth.

[19] Figure 10 shows the growth of BxPC3 tumor cells in mice treated with either IMC-11F8 or IMC-C225 (CETUXIMAB). Untreated animals serve as control for tumor growth.

DETAILED DESCRIPTION OF THE INVENTION

[20] The present invention provides human antibodies, and fragments thereof, specific for EGFR, as well as isolated or purified polynucleotide sequences encoding the

antibodies. Antibodies of the present invention can be used to treat neoplastic diseases, including solid and non-solid tumors and for treatment of hyperproliferative disorders.

[21] Naturally occuring antibody typically have two identical heavy chains and two identical light chains, with each light chain covalently linked to a heavy chain by an interchain disulfide bond and multiple disulfide bonds further link the two heavy chains to one another. Individual chains can fold into domains having similar sizes (110-125 amino acids) and structures, but different functions. The light chain can comprise one variable domain (V_L) and/or one constant domain (C_L). The heavy chain can also comprise one variable domain (V_H) and/or, depending on the class or isotype of antibody, three or four constant domains (C_H1, C_H2, C_H3 and C_H4). In humans, the isotypes are IgA, IgD, IgE, IgG, and IgM, with IgA and IgG further subdivided into subclasses or subtypes (IgA_{1-2} and IgG_{1-4}).

[22] Generally, the variable domains show considerable amino acid sequence variability from one antibody to the next, particularly at the location of the antigen-binding site. Three regions, called hypervariable or complementarity-determining regions (CDRs), are found in each of V_L and V_H, which are supported by less variable regions called framework variable regions.

[23] The portion of an antibody consisting of V_L and V_H domains is designated Fv (Fragment variable) and constitutes the antigen-binding site. Single chain Fv (scFv) is an antibody fragment containing a V_L domain and a V_H domain on one polypeptide chain, wherein the N terminus of one domain and the C terminus of the other domain are joined by a flexible linker (see, e.g., U.S. Pat. No. 4,946,778 (Ladner et al.); WO 88/09344, (Huston et al.). WO 92/01047 (McCafferty et al.) describes the display of scFv fragments on the surface of soluble recombinant genetic display packages, such as bacteriophage.

[24] The peptide linkers used to produce the single chain antibodies can be flexible peptides selected to assure that the proper three-dimensional folding of the V_L and V_H

domains occurs. The linker is generally 10 to 50 amino acid residues. Preferably, the linker is 10 to 30 amino acid residues. More preferably the linker is 12 to 30 amino acid residues. Most preferably is a linker of 15 to 25 amino acid residues. An example of such linker peptides includes $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ (SEQ ID NO:19).

[25] Single chain antibodies lack some or all of the constant domains of the whole antibodies from which they are derived. Therefore, they can overcome some of the problems associated with the use of whole antibodies. For example, single-chain antibodies tend to be free of certain undesired interactions between heavy-chain constant regions and other biological molecules. Additionally, single-chain antibodies are considerably smaller than whole antibodies and can have greater permeability than whole antibodies, allowing single-chain antibodies to localize and bind to target antigen-binding sites more efficiently. Furthermore, the relatively small size of single-chain antibodies makes them less likely to provoke an unwanted immune response in a recipient than whole antibodies.

[26] Multiple single chain antibodies, each single chain having one V_H and one V_L domain covalently linked by a first peptide linker, can be covalently linked by at least one or more peptide linker to form a multivalent single chain antibodies, which can be monospecific or multispecific. Each chain of a multivalent single chain antibody includes a variable light chain fragment and a variable heavy chain fragment, and is linked by a peptide linker to at least one other chain. The peptide linker is composed of at least fifteen amino acid residues. The maximum number of amino acid residues is about one hundred.

[27] Two single chain antibodies can be combined to form a diabody, also known as a bivalent dimer. Diabodies have two chains and two binding sites, and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to a V_L domain. The domains are connected with linkers that are short enough to prevent

pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites.

[28] Three single chain antibodies can be combined to form triabodies, also known as trivalent trimers. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain directly fused to the carboxyl terminus of a V_L or V_H domain, i.e., without any linker sequence. The triabody has three Fv heads with the polypeptides arranged in a cyclic, head-to-tail fashion. A possible conformation of the triabody is planar with the three binding sites located in a plane at an angle of 120 degrees from one another. Triabodies can be monospecific, bispecific or trispecific.

[29] Fab (Fragment, antigen binding) refers to the fragments of the antibody consisting of V_L C_L V_H C_{H1} domains. Those generated following papain digestion simply are referred to as Fab and do not retain the heavy chain hinge region. Following pepsin digestion, various Fabs retaining the heavy chain hinge are generated. Those fragments with the interchain disulfide bonds intact are referred to as $F(ab')_2$, while a single Fab' results when the disulfide bonds are not retained. $F(ab')_2$ fragments have higher avidity for antigen that the monovalent Fab fragments.

[30] Fc (Fragment crystallization) is the designation for the portion or fragment of an antibody that comprises paired heavy chain constant domains. In an IgG antibody, for example, the Fc comprises C_{H2} and C_{H3} domains. The Fc of an IgA or an IgM antibody further comprises a C_{H4} domain. The Fc is associated with Fc receptor binding, activation of complement-mediated cytotoxicity and antibody-dependent cellular-cytoxicity (ADCC). For antibodies such as IgA and IgM, which are complexes of multiple IgG like proteins, complex formation requires Fc constant domains.

[31] Finally, the hinge region separates the Fab and Fc portions of the antibody, providing for mobility of Fabs relative to each other and relative to Fc, as well as including multiple disulfide bonds for covalent linkage of the two heavy chains.

[32] Thus, antibodies of the invention include, but are not limited to, naturally occurring antibodies, bivalent fragments such as $(Fab')_2$, monovalent fragments such as Fab, single chain antibodies, single chain Fv (scFv), single domain antibodies, multivalent single chain antibodies, diabodies, triabodies, and the like that bind specifically with antigens.

[33] The antibodies, or fragments thereof, of the present invention are specific for EGFR. Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Antibodies, or fragments thereof, of the present invention, for example, can be monospecific or bispecific. Bispecific antibodies (BsAbs) are antibodies that have two different antigen-binding specificities or sites. Where an antibody has more than one specificity, the recognized epitopes can be associated with a single antigen or with more than one antigen. Thus, the present invention provides bispecific antibodies, or fragments thereof, that bind to two different antigens, with at least one specificity for EGFR.

[34] Specificity of the present antibodies, or fragments thereof, for EGFR can be determined based on affinity and/or avidity. Affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (K_d), measures the binding strength between an antigenic determinant and an antibody-binding site. Avidity is the measure of the strength of binding between an antibody with its antigen. Avidity is related to both the affinity between an epitope with its antigen binding site on the antibody, and the valence of the antibody, which refers to the number of antigen binding sites of a particular epitope. Antibodies typically bind with a dissociation constant (K_d) of 10^{-5} to 10^{-11} liters/mol. Any K_d less than 10^{-4} liters/mol is generally considered to indicate nonspecific binding. The lesser the value of the K_d, the stronger the binding strength between an antigenic determinant and the antibody binding site.

[35] Antibodies of the present invention, or fragments thereof, also include those for which binding characteristics have been improved by direct mutation, methods of affinity

maturation, phage display, or chain shuffling. Affinity and specificity can be modified or improved by mutating CDRs and screening for antigen binding sites having the desired characteristics (see, e.g., Yang et al., J. Mol. Biol., (1995) 254: 392-403). CDRs are mutated in a variety of ways. One way is to randomize individual residues or combinations of residues so that in a population of, otherwise identical antigen binding sites, all twenty amino acids are found at particular positions. Alternatively, mutations are induced over a range of CDR residues by error prone PCR methods (see, e.g., Hawkins et al., J. Mol. Biol., (1992) 226: 889-896). For example, phage display vectors containing heavy and light chain variable region genes can be propagated in mutator strains of *E. coli* (see, e.g., Low et al., J. Mol. Biol., (1996) 250: 359-368). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

[36] Equivalents of the antibodies, or fragments thereof, of the present invention include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the full-length anti-EGFR antibodies. Substantially the same amino acid sequence is defined herein as a sequence with at least 70%, preferably at least about 80%, and more preferably at least about 90% homology to another amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman (Proc. Natl. Acad. Sci. USA (1988) 85, 2444-8).

[37] The antibodies of the present invention, or fragments thereof, are human antibodies having one, two, three, four, five, and/or six complementarity determining regions (CDRs) selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13. Preferably, the antibodies (or fragments thereof) of the present invention have CDRs of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5. Alternatively and also preferably, the present antibodies, or fragments thereof, have CDRs of SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:13. The amino acid sequences of the CDRs are set forth below in Table 1.

TABLE 1

Heavy Chain		
CDR1	SGDYYWS	SEQ ID NO:1
CDR2	YIYYSGSTDYNPSLKS	SEQ ID NO:3
CDR3	VSIFGVGTFDY	SEQ ID NO:5
Light Chain		
CDR1	RASQSVSSYLA	SEQ ID NO:9
CDR2	DASNRAT	SEQ ID NO:11
CDR3	HQYGSTPLT	SEQ ID NO:13

[38] In another embodiment, the present antibodies, or fragments thereof, can have a heavy chain variable region of SEQ ID NO:7 and/or a light chain variable region of SEQ ID NO:15. IMC-11F8 is a particularly preferred antibody of the present invention. This antibody has human V_H and V_L framework regions (FWs) as well as CDRs. The V_H variable domain of IMC-11F8 (SEQ ID NO:7) has three CDRs (SEQ ID NOs:1, 3 and 5) and three FWs and the V_L domain (SEQ ID NO:15) has three CDRs (SEQ ID NOs:9, 11 and 13) and three FWs.

[39] Each domain of the antibodies of this invention can be a complete antibody with the heavy or light chain variable domain, or it can be a functional equivalent or a mutant or derivative of a naturally-occurring domain, or a synthetic domain constructed, for example, *in vitro* using a technique such as one described in WO 93/11236 (Griffiths et al.). For instance, it is possible to join together domains corresponding to antibody variable domains, which are missing at least one amino acid. The important characterizing feature is the ability of each domain to associate with a complementary domain to form an antigen-binding site. Accordingly, the terms variable heavy and light chain fragment should not be construed to exclude variants that do not have a material effect specificity.

[40] Preferably, the antibodies, or fragments thereof, of the present invention neutralize EGFR. Binding of a ligand, e.g., EGFor TGF-α, to an external, extracellular domain of EGFR stimulates receptor dimerization, autophosphorylation of EGFR, activation of the receptor's internal, cytoplasmic tyrosine kinase domain, and initiation of multiple signal transduction and transactivation pathways involved in regulation of DNA synthesis (gene activation) and cell cycle progression or division. Also preferably, the anti-EGFR antibodies (or fragments thereof) of the present invention are specific for the extracellular region of EGFR. The present antibodies, or fragments thereof, further preferably prevent binding of a ligand of EGFR to its receptor. In this embodiment, the antibodies of the present invention, or fragments thereof, bind EGFR at least as strongly as the natural ligands of EGFR (EGF and TNF-α).

[41] Neutralization of EGFR includes inhibition, diminution, inactivation and/or disruption of one or more of these activities normally associated with signal transduction. Thus, neutralizing EGFR has various effects, including inhibition, diminution, inactivation and/or disruption of growth (proliferation and differentiation), angiogenesis (blood vessel recruitment, invasion, and metastasis), and cell motility and metastasis (cell adhesion and invasiveness).

[42] One measure of EGFR neutralization is inhibition of the tyrosine kinase activity of the receptor. Tyrosine kinase inhibition can be determined using well-known methods; for example, by measuring the autophosphorylation level of recombinant kinase receptor, and/or phosphorylation of natural or synthetic substrates. Thus, phosphorylation assays are useful in determining neutralizing antibodies in the context of the present invention. Phosphorylation can be detected, for example, using an antibody specific for phosphotyrosine in an ELISA assay or on a western blot. Some assays for tyrosine kinase activity are described in Panek et al., *J. Pharmacol. Exp. Thera.* (1997) 283: 1433-44 and Batley et al., *Life Sci.* (1998) 62: 143-50.

[43] In addition, methods for detection of protein expression can be utilized to determine EGFR neutralization, wherein the proteins being measured are regulated by EGFR tyrosine kinase activity. These methods include immunohistochemistry (IHC) for detection of protein expression, fluorescence *in situ* hybridization (FISH) for detection of gene amplification, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern and Southern blots, reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA. *See, e.g.*, Grandis et al., Cancer, (1996) 78:1284-92; Shimizu et al., Japan J. Cancer Res., (1994) 85:567-71; Sauter et al., Am. J. Path., (1996) 148:1047-53; Collins, Glia, (1995) 15:289-96; Radinsky et al., Clin. Cancer Res., (1995) 1:19-31; Petrides et al., Cancer Res., (1990) 50:3934-39; Hoffmann et al., Anticancer Res., (1997) 17:4419-26; Wikstrand et al., Cancer Res., (1995) 55:3140-48.

[44] *In vivo* assays can also be utilized to determine EGFR neutralization. For example, receptor tyrosine kinase inhibition can be observed by mitogenic assays using cell lines stimulated with receptor ligand in the presence and absence of inhibitor. For example, A431cells (American Type Culture Collection (ATCC), Rockville, MD) stimulated with EGF can be used to assay EGFR inhibition. Another method involves testing for inhibition of growth of EGFR-expressing tumor cells, using for example, human tumor cells injected into a mouse. See U.S. Patent No. 6,365,157 (Rockwell et al.)

[45] The present invention is not limited by any particular mechanism of EGFR neutralization. The anti-EGFR antibodies of the present invention can bind externally to the EGF cell surface receptor, block binding of ligand (e.g., EGF or TGF-α) and subsequent signal transduction mediated via the receptor-associated tyrosine kinase, and prevent phosphorylation of the EGFR and other downstream proteins in the signal transduction cascade. The receptor-antibody complex can also be internalized and degraded, resulting in receptor cell surface downregulation. Matrix metalloproteinases, which function in tumor cell invasion and metastasis, can also be downregulated by the

antibodies of the present invention. Moreover, antibodies of the present invention may exhibit inhibition of growth factor production and angiogenesis.

[46] The antibodies of the present invention may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein, Nature, 256: 495-497 (1975) and Campbell, Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas, Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al., Science, 246, 1275-1281 (1989).

[47] Antibody fragments can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi et al., *J. Immunol. Methods*, 56: 235-243 (1983) and by Parham, *J. Immunol.* 131: 2895-2902 (1983). Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, i.e. scFv, dibodies, or other antibody fragments. Methods of producing such functional equivalents are disclosed in PCT Application WO 93/21319, European Patent Application No. 239,400; PCT Application WO 89/09622; European Patent Application 338,745; and European Patent Application EP 332,424.

[48] Preferred host cells for transformation of vectors and expression of the receptor antagonists of the present invention are mammalian cells, e.g., COS-7 cells, chinese hamster ovary (CHO) cells, and cell lines of lymphoid origin such as lymphoma, myeloma, or hybridoma cells. Other eukaryotic host, such as yeasts, can be alternatively used. For example, mouse fetal liver stromal cell line 2018 binds APtag-flk 1 and APtag-flk-2 fusion proteins, i.e., contains ligands of VEGFR-2 and flk-2 (ATCC, Manassas, VA, CRL 10907), human fetal spleen cell line Fsp 62891 contains flk-2 ligand (ATCC CRL

10935), and human stromal fetal thymus cell line, F.thy 62891, contains VEGFR-2 ligand (ATCC CRL 10936).

[49] The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon (carbohydrates such as glucose or lactose), nitrogen (amino acids, peptides, proteins or their degradation products such as peptones, ammonium salts or the like), and inorganic salts (sulfates, phosphates and/or carbonates of sodium, potassium, magnesium and calcium). The medium furthermore contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like.

[50] Where it is desired to express a gene construct in yeast, a suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7. Stinchcomb et al. *Nature*, 282: 39 (1979); Kingsman et al., *Gene*, 7: 141 (1979). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85: 12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

[51] As described in the examples below, high affinity anti-EGFR antibodies according to the present invention can be isolated from a phage display library constructed from human heavy chain and light chain variable region genes. For example, a variable domain of the invention can be obtained from a peripheral blood lymphocyte that contains a rearranged variable region gene. Alternatively, variable domain portions, such as CDR and FW regions, can be derived from different human sequences.Over 90% of recovered clones after three rounds of selection are specific to EGFR. The binding affinities for

EGFR of the screened Fabs are in the nM range, which are as high as those of several bivalent anti-EGFR monoclonal antibodies produced using hybridoma technology.

[52] Antibodies, and fragments thereof, of the present invention can be obtained, for example, from naturally occurring antibodies, or Fab or scFv phage display libraries. It is understood that, to make a single domain antibody from an antibody comprising a V_H and a V_L domain, certain amino acid substitutions outside the CDRs can be desired to enhance binding, expression or solubility. For example, it can be desirable to modify amino acid residues that would otherwise be buried in the V_H-V_L interface.

[53] The protein used to identify EGFR binding antibodies of the invention is preferably EGFR and, more preferably, is the extracellular domain of EGFR. The EGFR extracellular domain can be free or conjugated to another molecule.

[54] The present invention also provides isolated polynucleotides encoding the antibodies, or fragments thereof, described previously. The invention includes nucleic acids having a sequence encoding one, two, three, four, five and/or all six CDRs. Table 2 sets forth the nucleic acid sequences.

TABLE 2

Heavy Chain		
CDR1	agtggtgatt actactggag t	SEQ ID NO:2
CDR2	tacatctatt acagtgggag caccgactac aacccgtccc tcaaagt	SEQ ID NO:4
CDR3	gtgtcgattt ttggagtggg ggacatttga ctac	SEQ IS NO:6
Light Chain		
CDR1	agggccagtc agagtgttag cagctactta gcc	SEQ ID NO:10
CDR2	gatgcatcca acagggccac t	SEQ ID NO:12
CDR3	caccagtatg gtagcacacc tctcact	SEQ ID NO:14

[55] DNA encoding human antibodies can be prepared by recombining DNA encoding human constant regions and variable regions, other than the CDRs, derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived from a human (SEQ ID NOs:2, 4 and 6 for the heavy chain variable domain CDRs and SEQ ID NOs:10, 12, 14 for the light chain variable domain CDRs).

[56] Suitable sources of DNAs that encode fragments of antibodies include any cell, such as hybridomas and spleen cells, that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above. The DNA deletions and recombinations described in this section may be carried out by known methods, such as those described in the published patent applications listed above in the section entitled "Functional Equivalents of Antibodies" and/or other standard recombinant DNA techniques, such as those described below. Another source of DNAs are single chain antibodies produced from a phage display library, as is known in the art.

[57] Additionally, the present invention provides expression vectors containing the polynucleotide sequences previously described operably linked to an expression sequence, a promoter and an enhancer sequence. A variety of expression vectors for the efficient synthesis of antibody polypeptide in prokaryotic, such as bacteria and eukaryotic systems, including but not limited to yeast and mammalian cell culture systems have been developed. The vectors of the present invention can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences.

[58] Any suitable expression vector can be used. For example, prokaryotic cloning vectors include plasmids from *E. coli*, such as *colE1*, *pCR1*, *pBR322*, *pMB9*, *pUC*, *pKSM*, and *RP4*. Prokaryotic vectors also include derivatives of phage DNA such as *M13* and other filamentous single-stranded DNA phages. An example of a vector useful in yeast is the 2μ plasmid. Suitable vectors for expression in mammalian cells include well-known

derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

[59] Additional eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet., 1, 327-341 (1982); Subramani et al., Mol. Cell. Biol., 1: 854-864 (1981); Kaufmann and Sharp, "Amplification And Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); Kaufmann and Sharp, Mol. Cell. Biol. 159, 601-664 (1982); Scahill et al., "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Nat'l Acad. Sci. USA 80, 4654-4659 (1983); Urlaub and Chasin, Proc. Nat'l Acad. Sci. USA 77, 4216-4220, (1980).

[60] The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

[61] The present invention also provides recombinant host cells containing the expression vectors previously described. Antibodies of the present invention can be

expressed in cell lines other than in hybridomas. Nucleic acids, which comprise a sequence encoding a polypeptide according to the invention, can be used for transformation of a suitable mammalian host cell.

[62] Cell lines of particular preference are selected based on high level of expression, constitutive expression of protein of interest and minimal contamination from host proteins. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others. Suitable additional eukaryotic cells include yeast and other fungi. Useful prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCl, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*.

[63] These present recombinant host cells can be used to produce an antibody, or fragment thereof, by culturing the cells under conditions permitting expression of the antibody or fragment thereof and purifying the antibody or fragment thereof from the host cell or medium surrounding the host cell. Targeting of the expressed antibody or fragment for secretion in the recombinant host cells can be facilitated by inserting a signal or secretory leader peptide-encoding sequence (see, Shokri et al., (2003) Appl Microbiol Biotechnol. 60(6):654-64, Nielsen et al., Prot. Eng. (1997) 10:1-6 and von Heinje et al., (1986) Nucl. Acids Res. 14:4683-4690) at the 5' end of the antibody-encoding gene of interest. These secretory leader peptide elements can be derived from either prokaryotic or eukaryotic sequences. Accordingly suitably, secretory leader peptides are used, being amino acids joined to the N-terminal end of a polypeptide to direct movement of the polypeptide out of the host cell cytosol and secretion into the medium.

[64] The antibodies of this invention can be fused to additional amino acid residues. Such amino acid residues can be a peptide tag, perhaps to facilitate isolation. Other amino

acid residues for homing of the antibodies to specific organs or tissues are also contemplated.

[65] Another embodiment for the preparation of antibodies in the present invention is the expression of the nucleic acid encoding the antibody according to the invention in a transgenic animal that has a substantial portion of the human antibody producing genome inserted and is rendered deficient in the production of endogenous antibodies. Transgenic animals, include but not limited to mice, goat, and rabbit. One further embodiment of the invention, include expression of the antibody-coding gene in, for example, the mammary gland of the animal for secretion of the polypeptide during lactation.

[66] A method of treating tumor growth in a mammal by administering to the mammal an effective amount of an antibody as previously described is also provided by the present invention. Suitable tumors to be treated according to the present invention preferably express EGFR. While not intended to be bound to any particular mechanism, the diseases and conditions which can be treated or prevented by the present methods include, for example, those in which pathogenic angiogenesis or tumor growth is stimulated through a EGFR paracrine and/or autocrine loop.

[67] EGFR expression has been observed in a variety of human tumors, both *in vitro* and *in vivo*, and the levels of EGFR expression vary widely with tumor type. EGFR is expressed at varying levels on the cell surface in a significant percentage of human tumors, such as colorectal, head and neck (squamous cell), pancreatic, lung, breast, and renal cell carcinomas, as well as glioblastoma. In certain tumor types, EGFR expression is very common (e.g., 35% to 70% of ovarian cancers and approximately 25% to 77% of colorectal cancers). High levels of EGFR expression can occur in correlation with production of receptor ligands (i.e., EGF and TGF-α). EGFR expression has also been correlated with increased resistance to certain chemotherapeutic agents and radiotherapy. EGFR expression may also serve as a prognostic factor in certain types of tumors, as it has

be associated with reduced survival, poor prognosis, and/or increased risk of metastasis. Moreover, increased EGFR expression exists in multiple tumor types.

[68] Tumors to be treated include primary tumors and metastatic tumors, as well as refractory tumors. Refractory tumors include tumors that fail to respond or are resistant to treatment with chemotherapeutic agents alone, antibodies alone, radiation alone or combinations thereof. Refractory tumors also encompass tumors that appear to be inhibited by treatment with such agents, but recur up to five years, sometimes up to ten years or longer after treatment is discontinued.

[69] Antibodies of the present invention are useful for treating tumors that express EGFR. Such tumors are characteristically sensitive to EGF present in their environment, and can further produced and be stimulated by EGF in an autocrine stimulatory loop. The method is therefore effective for treating a solid or non-solid tumor that is not vascularized, or is not yet substantially vascularized. Examples of solid tumors, which can be accordingly treated, include breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma. Some examples of such tumors include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, including small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, and liver tumors. Other examples include Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, and leiomyosarcoma. Examples of vascularized skin cancers for which the antagonists of this invention are effective include squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes.

[70] Examples of non-solid tumors include leukemia, multiple myeloma and lymphoma. Some examples of leukemias include acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), erythrocytic leukemia or monocytic leukemia. Some examples of lymphomas include Hodgkin's and non-Hodgkin's lymphoma.

[71] In another aspect of the invention, anti-EGFR antibodies are used to inhibit angiogenesis. EGFR stimulation of vascular endothelium is associated with angiogenic diseases and vascularization of tumors. Typically, vascular endothelium is stimulated in a paracrine fashion by EGF from other sources (e.g., tumor cells).

[72] Accordingly, the human anti-EGFR antibodies are effective for treating subjects with vascularized tumors or neoplasms or angiogenic diseases. Such tumors and neoplasms include, for example, malignant tumors and neoplasms, such as blastomas, carcinomas or sarcomas, and highly vascular tumors and neoplasms. Cancers that can be treated by the methods of the present invention include, for example, cancers of the brain, genitourinary tract, lymphatic system, stomach, renal, colon, larynx and lung and bone. Non-limiting examples further include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, including lung adenocarcinoma and small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, and liver tumors. The method is also used for treatment of vascularized skin cancers, including squamous cell carcinoma, basal cell carcinoma, and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes. Other cancers that can be treated include Kaposi's sarcoma, CNS neoplasms (neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases), melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, including glioblastoma multiforme, and leiomyosarcoma.

[73] A further aspect of the present invention includes methods of treating or preventing pathologic conditions characterized by excessive angiogenesis or a non-cancer hyperproliferative cell growth, involving, for example, vascularization and/or inflammation, such as atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, and psoriasis. Other non-limiting examples of non-neoplastic angiogenic disease are retinopathy of prematurity (retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, Crohn's disease, autoimmune nephritis, primary biliary cirrhosis, acute pancreatitis, allograft rejection, allergic inflammation, contact dermatitis and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restenosis, and fungal, parasitic and viral infections, including cytomegaloviral infections.

[74] The present invention also provides a method of treating a non-cancer hyperproliferative disease in a mammal comprising administering to the mammal an effective amount of the antibody of the present invention.

[75] In the methods of the present invention, a therapeutically effective amount of an antibody of the invention is administered to a mammal in need thereof. The term administering as used herein means delivering the antibodies of the present invention to a mammal by any method that can achieve the result sought. They can be administered, for example, intravenously or intramuscularly. Although human antibodies of the invention are particularly useful for administration to humans, they can be administered to other mammals as well. The term mammal as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. Therapeutically effective amount means an amount of antibody of the present invention that, when

administered to a mammal, is effective in producing the desired therapeutic effect, such as inhibiting kinase activity or inhibition of tumor growth.

[76] The identification of such disease is well within the ability and knowledge of one skilled in the art. For example, human individuals who are either suffering from a clinically significant neoplastic or angiogenic disease or who are at risk of developing clinically significant symptoms are suitable for administration of the present EGFR antibodies. A clinician skilled in the art can readily determine, for example, by the use of clinical tests, physical examination and medical/family history, if an individual is a candidate for such treatment.

[77] The present anti-EGFR antibodies can be administered for therapeutic treatments to a patient suffering from a tumor or angiogenesis associated pathologic condition in an amount sufficient to prevent, inhibit, or reduce the progression of the tumor or pathologic condition. Progression includes, e.g., the growth, invasiveness, metastases and/or recurrence of the tumor or pathologic condition. An amount adequate to accomplish this is defined as a therapeutically effective dose. Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's own immune system. Dosing schedules will also vary with the disease state and status of the patient, and will typically range from a single bolus dosage or continuous infusion to multiple administrations per day (e.g., every 4-6 hours), or as indicated by the treating physician and the patient's condition. It should be noted, however, that the present invention is not limited to any particular dose.

[78] A cocktail of EGFR antagonists, e.g., monoclonal antibodies, provides an especially efficient treatment for inhibiting the growth of tumor cells. The cocktail can include non-antibody EGFR antagonists and can have as few as 2, 3 or 4 receptor antagonists, and as many as 6, 8 or 10.

[79] In an embodiment of the invention, anti-EGFR antibodies can be administered in combination with one or more other anti-neoplastic agents. For examples of combination therapies, see, e.g., U.S. Patent No. 6,217,866 (Schlessinger et al.) (Anti-EGFR antibodies in combination with anti-neoplastic agents); WO 99/60023 (Waksal et al.) (Anti-EGFR antibodies in combination with radiation). Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation or combinations thereof. The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but not limited to, doxorubicin, daunorubicin, paclitaxel, irinotecan (CPT-11), and topotecan. When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity tumor being treated and the route of administration of the agent. It should be emphasized, however, that the present invention is not limited to any particular dose.

[80] Further, anti-EGFR antibodies of the invention can be administered with antibodies that neutralize other receptors involved in tumor growth or angiogenesis. In an embodiment of the invention, an anti-EGFR antibody is used in combination with a receptor antagonist that binds specifically to EGFR. Particularly preferred are antigen-binding proteins that bind to the extracellular domain of EGFR and block binding of one or more of its ligands and/or neutralize ligand-induced activation of EGFR. An EGFR antagonist can be an antibody that binds to EGFR or a ligand of EGFR and inhibits binding of EGFR to its ligand. Ligands for EGFR include, for example, EGF, TGF-α amphiregulin, heparin-binding EGF (HB-EGF) and betacellulin. EGF and TGF-α are thought to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. It should

be appreciated that the EGFR antagonist can bind externally to the extracellular portion of EGFR, which can or can not inhibit binding of the ligand, or internally to the tyrosine kinase domain. Examples of EGFR antagonists that bind EGFR include, without limitation, biological molecules, such as antibodies (and functional equivalents thereof) specific for EGFR, and small molecules, such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR.

[81] Another example of such a receptor is VEGFR. In an embodiment of the present invention, an anti-EGFR antibody is used in combination with a VEGFR antagonist. In one embodiment of the invention, an anti-EGFR antibody is used in combination with a receptor antagonist that binds specifically to VEGFR-1/Flt-1 receptor. Particularly preferred are antigen-binding proteins that bind to the extracellular domain of VEGFR-1 and block binding by one or both of its ligands, VEGF and PIGF, and/or neutralize VEGF-induced or PIGF-induced activation of VEGFR-1. For example, MAb 6.12 is a scFv that binds to soluble and cell surface-expressed VEGFR-1. ScFv 6.12 comprises the V_L and V_H domains of mouse monoclonal antibody MAb 6.12. A hybridoma cell line producing MAb 6.12 has been deposited as ATCC number PTA-3344 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty).

[82] Other examples of growth factor receptors involved in tumorigenesis are the receptors for platelet-derived growth factor (PDGFR), insulin-like growth factor (IGFR), nerve growth factor (NGFR), and fibroblast growth factor (FGFR).

[83] In an additional alternative embodiment, the EGFR antagonist can be administered in combination with one or more suitable adjuvants, such as, for example, cytokines (IL-10 and IL-13, for example) or other immune stimulators, such as, but not limited to, chemokine, tumor-associated antigens, and peptides. See, e.g., Larrivée et al., supra. It should be appreciated, however, that administration of only an anti-EGFR antibody is

sufficient to prevent, inhibit, or reduce the progression of the tumor in a therapeutically effective manner.

[84] In a combination therapy, the anti-EGFR antibody is administered before, during, or after commencing therapy with another agent, as well as any combination thereof, i.e., before and during, before and after, during and after, or before, during and after commencing the anti-neoplastic agent therapy. For example, the anti-EGFR antibody can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered concurrently with or, more preferably, subsequent to antibody therapy.

[85] In the present invention, any suitable method or route can be used to administer anti-EGFR antibodies of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

[86] It is noted that an anti-EGFR antibody of the invention can be administered as a conjugate, which binds specifically to the receptor and delivers a toxic, lethal payload following ligand-toxin internalization. The antibody-drug/small molecule conjugate can be directly linked to each other or via a linker, peptide or non-peptide.

[87] In another aspect of the invention, an anti-EGFR antibody of the invention can be chemically or biosynthetically linked to one or more anti-neoplastic or anti-angiogenic agents.

[88] The invention further contemplates anti-EGFR antibodies to which target or reporter moieties are linked. Target moieties are first members of binding pairs. Anti-neoplastic agents, for example, are conjugated to second members of such pairs and are thereby directed to the site where the anti-EGFR antibody is bound. A common example of such a binding pair is avidin and biotin. In a preferred embodiment, biotin is conjugated to an anti-EGFR antibody, and thereby provides a target for an anti-neoplastic agent or other moiety, which is conjugated to avidin or streptavidin. Alternatively, biotin or another such moiety is linked to an anti-EGFR antibody of the invention and used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin.

[89] It is understood that the anti-EGFR antibodies of the invention, where used in a mammal for the purpose of prophylaxis or treatment, will be administered in the form of a composition additionally comprising a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Pharmaceutically acceptable carriers can further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the binding proteins. The compositions of the injection can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

[90] The present invention also includes kits for inhibiting tumor growth and/or angiogenesis comprising a therapeutically effective amount of a human anti-EGFR antibody. The kits can further contain any suitable antagonist of, for example, another

growth factor receptor involved in tumorigenesis or angiogenesis (e.g., VEGFR-1/Flt-1, VEGFR-2, PDGFR, IGFR, NGFR, FGFR, etc, as described above). Alternatively, or in addition, the kits of the present invention can further comprise an anti-neoplastic agent. Examples of suitable anti-neoplastic agents in the context of the present invention have been described herein. The kits of the present invention can further comprise an adjuvant; examples have also been described above.

[91] Moreover, included within the scope of the present invention is use of the present antibodies *in vivo* and *in vitro* for investigative or diagnostic methods, which are well known in the art. The diagnostic methods include kits, which contain antibodies of the present invention.

[92] Accordingly, the present receptor antagonists thus can be used *in vivo* and *in vitro* for investigative, diagnostic, prophylactic, or treatment methods, which are well known in the art. Of course, it is to be understood and expected that variations in the principles of invention herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

[93] Increased EGFR activation is sometimes associated with the conditions that are treated according to the present invention. Higher levels of ligand, EGFR gene amplification, increased transcription of the receptor or mutations that cause unregulated receptor signaling can result in increased EGFR activation. Amplification of the gene encoding EGFR also results in an increased number of ligands binding to the EGFR, which can further stimulate cell proliferation. EGFR may be overexpressed in the absence of gene amplification, presumably through mutations that increase EGFR transcription, mRNA translation, or stability of the protein. EGFR mutants have been identified in gliomas, non-small-cell lung carcinomas, ovarian carcinomas and prostate carcinomas that have a constitutively active tyrosine kinase, suggesting a role for high-level EGFR activity rather than EGFR overexpression in these cancers. *See, e.g.*, Pedersen et al., *Ann. Oncol.*,

12(6):745-60 (2001). (Type III EGFR mutation – variously named EGFRvIII, de2-7 EGFR or AEGFR – lacks a portion of the extracellular ligand binding domain encoded by exons 2-7.); *see also* Wikstrand et al., *Cancer Res.*, 55:3140-3148 (1995).

EXAMPLES

[94] The following examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press. All references mentioned herein are incorporated in their entirety.

EGFR-Expressing Cells

[95] NIH 3T3 cells were obtained from the ATCC (Rockville, MD) and were transfected with human EGFR cDNA. The resulting cell line was maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum (CS), 1 mM L-glutamine, antibiotics, and 600 μg/mL G418 (Geneticin; Sigma, St. Louis, MO).

Example 1

Isolation of Human Anti-EGFR Antibodies

[96] Briefly, the human antibodies were isolated from a human Fab library (containing the heavy and light chain variable regions of the antibody producing cells of human donors) by biopanning against human EGFR isolated from EGFR-positive tumors.

[97] The Fab library stock was grown to log phase, rescued with M13K07 helper phage and amplified overnight in 2YTAK medium (2YT containing 100 μg/ml of ampicillin and 50 μg/ml of kanamycin) at 30°C. The phage preparation was precipitated in 4% PEG/0.5M NaCl, resuspended in 3% fat-free milk/PBS containing 500 μg/ml of alkaline phosphatase (AP) and incubated at 37°C for 1 h to block phage-scFv having specificity for AP scFv and to block other nonspecific binding.

[98] Human EGFR from EGFR-expressing tumors (10 μg/ml) coated Maxisorp Star tubes (Nunc, Denmark) were first blocked with 3% milk/PBS at 37°C for 1 h, and then incubated with the phage preparation at room temperature for 1 h. The tubes were washed 10 times with PBST (PBS containing 0.1% Tween 20), followed by 10 times with PBS. The bound phage were eluted at room temperature for 10 minutes with 1 ml of a freshly prepared solution of 100 mM triethylamine. The eluted phages were incubated with 10 ml of mid-log phase TG1 cells at 37°C for 30 minutes stationary and 30 minutes shaking. The infected TG1 cells were then plated onto 2YTAG plates and incubated overnight at 30°C.

[99] Successive rounds of the screening procedure were employed to further enrich for displayed Fab having the desired binding specificity. After two or three rounds of panning, individual bacterial colonies were screened individually to identify clones having desired EGFR binding characteristics. Identified clones were further tested for blocking of EGF binding. DNA fingerprinting of clones was used to differentiate unique clones. Representative clones of each digestion pattern were picked and subject to DNA sequencing.

Example 2

Construction of Human Anti-EGFR IgG1 Antibodies

[100] The human anti-EGFR Fab is engineered into a full human IgG1.

Example 3

Generation of Human Anti-EGFR IgG1 Antibodies

[101] The IgG1 antibody was expressed and purifed from *E. coli* strain, NSO/GS system containing an expression plasmid grown at 30° C in a shaker flask. *See, e.g.*, Lu et al., *J. Immunol. Methods* (1999) 230:159-171.

[102] A periplasmic extract of the cells was then prepared by resuspending the cell pellet in 25 mM Tris (pH 7.5) containing 20% (w/v) sucrose, 200 mM NaCl, 1 mM EDTA and 0.1 mM PMSF, followed by incubation at 4°C with gentle shaking for 1 h. After centrifugation at 15,000 rpm for 15 minutes, the soluble IgG1 antibody was purified from the supernatant by anti-E tag affinity chromatography using the RPAS Purification Module (Amersham Pharmacia Biotech). To examine the purity of the antibody preparation, both the *E. coli* periplasmic extract and the purified diabody were electrophoresed in an 18% polyacrylamide gel (Novex, San Diego, CA) and visualized by staining with Colloidal Blue Stain kit (Novex).

Example 4

In Vitro Binding of Antibodies to EGFR.

[103] Antibodies were screened in a solid state ELISA comparing the binding characteristics of IMC-11F8 and IMC-C225. Ninety six-well microtiter plate was coated overnight with 1 μg/mL in carbonate buffer at 4°C. Plates were blocked with phosphate buffered saline (PBS) supplemented with 10% new born calf serum for one hour at 37°C. Various amounts of IMC-11F8 or IMC-C225 were added to the plates and incubated at room temperature for a further 60 minutes, followed by washing with PBS. Mouse anti-human Fc antibody-horse radish peroxidase (HRP) conjugate were added and incubated for an additional 60 minutes at room temperature, followed by extensive washing with PBS. The plate was then incubated with HRP substrate for (**HOW LONG**) and the

reaction stopped with (**CONCENTRATION USED**) H_2SO_4. The plates were read using an ELISA reader at OD_{450nm}.

[104] Figure 1 shows the binding of IMC-11F8 and IMC-C225 antibodies to EGFR. Both IMC-11F8 and IMC-C225 binds equally well to EGFR.

Example 5

Binding Kinetics of Anti-EGFR Antibodies

[105] The binding kinetics of IMC-11F8 and IMC-C225 IgG antibodies and their respective Fab fragments were measured using a BIAcore sensor (Pharmacia Biosensor,) EGFR-AP fusion protein was immobilized onto a sensor chip and soluble IMC-11F8 and IMC-C225 antibodies were injected at concentrations ranging from 1.5 nM to 100 nM. Sensorgrams were obtained at each concentration and were analyzed with, BIA Evaluation 2.0, a program to determine the rate constants, k_{on} and k_{off}. The affinity constant, K*d*, was calculated from the ratio of rate constants, k_{off}/k_{on}.

[106] The binding kinetics of the anti-EGFR antibodies of the present invention are illustrated in Table 3. These show that both IgG antibodies have comparable binding kinetics to EGFR.

TABLE 3

Antibody	Format	k_{on} (10^5 $M^{-1}s^{-1}$)	k_{off} (10^{-4} s^{-1})	K_d (nM)
IMC-11F8	Fab	22.9 ± 9.9	36.7 ± 8.5	1.78 ± 0.5
IMC-11F8	IgG	18.6 ± 7.7	5.8 ± 2.2	0.32 ± 0.06
IMC-C225	Fab	23.1 ± 4.8	11.7 ± 3.4	0.53 ± 0.17
IMC-C225	IgG	21.3 ± 7.3	5.4 ± 1.0	0.3 ± 0.2

The results represent the mean ± SE from at least three separate determinations.

Example 6

Specificity of the Antibodies for EGFR

[107] Antibody binding to EGFR was evaluated by a ^{125}I-EGF competition assay. HT29 cells were seeded at 2 x 10^4 cells per well in 24-well COSTAR™ plates (Fisher Scientific, U.S.A.) in McCoy's 5a medium supplemented with 1.5 mM L-glutamine, 10% CS and antibiotics at 37°C. The cell monolayer was then incubated at room temperature for 1 hour with various concentrations of unlabeled EGF, 11F8 or IMC-C225 that were mixed with various amounts of ^{125}I-labeled EGF. Cells were washed with cold PBS and cell-associated radioactivity was measured in a gamma counter.

[108] Figure 2 shows the inhibition of ^{125}I-EGF binding to EGFR on HT29 cells. At concentrations of between 10 to 100nM, IMC-11F8 is as efficient as IMC-C225 in inhibiting ^{125}I-EGF binding to EGFR on HT29 cells. Both antibodies are better at competing for binding than EGF, the natural ligand of EGFR.

Example 7

EGFR Activation

[109] Briefly, a kinase receptor activation assay (KIRA assay), or phosphorylation assay, was carried out using BxPC3 or A431 cells. Cells were first grown to 90% confluency in DME supplemented with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 10% CS, at 37°C. Prior to experimentation, the cells were starved for 24 h in DME supplemented with 0.5% CS. To evaluate the effects of antibodies, IMC-11F8, IMC-C225 and IMC-1C11 on EGF-induced activation of EGFR, various concentrations of antibodies were prebound at room temperature for 30 minutes, followed by stimulation with EGF at 8 ng/mL for another 15 minutes. Following stimulation, cell monolayers were washed with ice cold PBS containing 1 mM sodium orthovanadate. Cells were lysed in lysis buffer [20 mM Tric-HCl, pH. 7.4, 1% Triton X-

100, 137 mM NaCl, 10% glycerol, 10 mM EDTA, 2 mM sodium orthovanadate, 100 mM NaF, 100 mM sodium pyrophosphate, 5 mM PEFABLOC® SC (Boehringer Mannheim Biochemicals, Indianapolis, IN), 100 μg aprotinin and 100 μg/mL leupeptin] and centrifuged at 14,000 x g for 10 minutes. Cleared cell lysates were added to wells of 96-well plates coated with polyclonal anti-EGFR antibody. The plates were washed to remove non-specifically bound proteins and the level of EGFR phosphorylation was assessed by the addition of anti-phosphotyrosine antibody. Upon extensive washing, the amount of bound anti-phoshotyrosine antibody was measure using an ELISA reader at OD_{450nm}.

[110] The results show a marked decrease in phosphorylation of EGFR by IMC-11F8 antibody in both BxPC3 (Figure 3) and A431 (Figure 4) cells tested as compared to control antibody, IMC-1C11.

[111] Inhibition of EGF-stimulated EGFR phosphorylation was further evaluated by Western blot analysis of the immunoprecipitated EGFR. A431 cells were prebound with antibodies followed by stimulation with EGF as described above. A control antibody that binds to EGFR but does not inhibit EGFR phosphorylation was used. Protein (EGFR) was immunoprecipitated from the cleared lysates using polyclonal anti-EGFR antibody followed by Protein A Sepharose beads. The bound-beads were then washed once with 0.2% Triton X-100, 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA (Buffer A), twice with Buffer A containing 500 mM NaCl and twice with Tris-HCl, pH 8.0. Drained beads were mixed with 30 μL 2 X SDS loading buffer, boiled and the supernatant was subjected to SDS-PAGE. After separation of proteins by electrophoresis, the protein bands were transferred onto nitrocellulose filters for Western Blot analysis. Filters were blocked overnight in blocking buffer, 50 mM Tris-HCl, pH7.4, 150 mM NaCl (TBS) containing 5% bovine serum albumin and 10% nonfat dried milk. To detect phophorylated receptor, blots were probed with an anti-phospphotyrosine antibody. in blocking buffer for 1 hour at room temperature. Blots were then washed extensively with

0.5 x TBS containing 0.1% Tween-20 (TBS-T) and incubated with goat anti-mouse Ig conjugated to HRP (Amersham, Little Chalfont, U.K.). Blots were washed with TBS and incubated for 1 minute with a chemiluminescence reagent (ECL, Amersham, Little Chalfont, U.K.). Anti-phosphotyrosine reacting with phosphorylated proteins was detected by exposure to a high performance luminescence detection film (Hyperfilm-ECL, Amersham, Little Chalfont, U.K.) for 0.5 to 10 minutes.

[112] Western blot analysis in Figure 5A shows that IMC-11F8, like IMC-C225, inhibits EGFR phosphorylation. Neither EGF- nor the control antibody-treated cells completely inhibits EGFR phosphorylation. Figure 5B and 6B show that synthesis of EGFR is not inhibited with the addition of antibodies to the cells. Figure 6A shows that phosphorylation of EGFR is inhibited when the concentration of IMC-11F8 at least 0.6 μg/mL was added to the cells.

Example 8

Inhibition of Cell Proliferation

[113] The MTT Cell Proliferation Assay is measured color-metrically as a result of reduction of the yellow tetrazolium, MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-phenyltetrazolium bromide) by metabolically active cell to an intracellular purple formazan product, which can be solubilized and quantified by spectrophotometric means. Briefly, DiFi cells were cultured overnight in DMEM-10% CS. Antibodies, IMC-11F8, IMC-C225 or IMC-1C11 were added to triplicate wells and incubated for an additional 72 hours at 37°C, 5% CO_2. To measure cell growth, a 20 μL aliquot of tetrazolium dye was added to each well and the cells were incubated for 3 hours at 37°C. When the purple precipitate was clearly visible under a microscope, the cells were lysed by addition of 100 μL detergent reagent. Absorbance of the formazan product was measure at OD_{570nm} as a quantitation of proliferation.

[114] Figure 7 shows that, unlike control antibody, IMC-1C11, IMC-11F8 is as potent an inhibitor of cell proliferation as IMC-C225.

Example 9

Antibody-Dependent Cellular Cytotoxicity (ADCC) Activity

[115] One method of assessing cell death is via an antibody dependent cell-mediated cytotoxicity assay or ADCC, which generally use the radioisotope ^{51}Cr. Target cells labeled with ^{51}Cr were mixed with antibody and the degree of killing was assessed by release of ^{51}Cr. Briefly, approximately 3 x 10^6 DiFi cells were suspended in 0.5 μL culture medium and 0.5 mCi of $Na^{51}CrO_4$ was added. The mixture was incubated for 1 h at 37°C with occasional shaking. The cells were then washed three times with cold culture medium. The labeled cells were then suspended in 100 μL culture medium containing varying concentrations of anti-EGFR antibodies (IMC-11F8 or IMC-C225) and incubated for 30 minutes at 4°C. The cells were then washed three times with culture medium by centrifugation. Rabbit complement was added and the treated cells were further incubated at 37°C for 1 h. Fifty μL of cold medium were then added and centrifuged. The supernatants were then removed and the radioactivity released by the cells into the supernatant was measured in a gamma counter. The maximum release of the radioactivity was obtained by adding 1% Triton X to the target cells. The percent cytotoxicity was calculated as cpm experimental release minus cpm background times 100%, which is then divided by the cpm maximum release minus cpm background.

[116] Figure 8 shows IMC-11F8 and IMC-C225 (or ERBITUX™) mediate cell death via activation of the complement lytic pathway in the cell (i.e., mediate Antibody Dependent Cellular Cytotoxicity or ADCC activity).

Example 10

In Vivo Inhibition of Tumor Cell Growth in Mice

[117] *In vivo* anti-tumor studies were designed to determine if IMC-11F8 would block the growth of tumor cells in a xenograft model. Athymic mice (nu/nu; Charles River Lab, Wilmington, MA) were injected subcutaneously with 1-2 million A431 or BxPC-3 cells in the flank. Anti-EGFR antibodies (IMC-11F8 and IMC-C225) or control antibody was administered intra-peritoneally at either 1 mg/dose or 0.3 mg/dose, three times per week. Tumor size was measured at least three times per week with a caliper and tumor volume calculated (*See*, e.g. Baselga et al., J Natl. Cancer Inst. (1993) 85:1327-1333)

[118] Figure 9 shows the anti-tumor activity of IMC-11F8 in A431 xenograft model. At 1 mg dose (Figure 9, right panel, IMC-11F8 is as effective as IMC-C225 (CETUXIMAB) in supressing or inhibiting tumor growth as compared to control animals. At a lower dose of 0.3 mg, progression of tumor growth is retarded. Similarly, Figure 10 shows the effect of IMC-11F8 and IMC-C225 in a second tumor model. The kinetics of BxPC3 tumor growth is similar to that observed in the A431 tumor model.

Example 11

[119] The pharmacokinetics of IMC-11F8 was studied in cynomolgus monkeys and compared to the pharmacokinetics of IMC-C225. A single dose pharmacokinetic study at 20.5 mg/kg ^{125}I-radio-labeled IMC-11F8 and IMC-C225 was separately injected intravenously in monkey and blood was drawn at day to determine the level of antibody that is retained in the plasma of the animal. Table 4 provides a pharmacokinetics comparison of IMC-11F8 and IMC-C225 in cynomolgus monkeys.

TABLE 4

	IMC-11F8	IMC-C225
C_{max} (mg/L)	1213	1161
T_{max} (hrs)	0.75	0.117
$T_{1/2}$ (hrs)	116	117
AUC (mg*hr/L)	115400	97871
CI (mL/hr)	0.736	0.636

[120] It is understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

ABSTRACT

The present invention provides a fully human antibody that binds human EGFR with affinity comparable to or higher than IMC-C225, and that neutralizes activation of EGFR. Antibodies include whole immunoglobulins, monovalent Fabs and single chain antibodies, multivalent single chains antibodies, diabodies, triabodies, and single domain antibodies. The invention further provides nucleic acids and host cells and animals that encode and express these antibodies. The invention further provides a method for neutralizing activation of EGFR, treating in a mammal with neoplastic growth and non-cancerous hyperproliferative diseases.

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FIG. 1

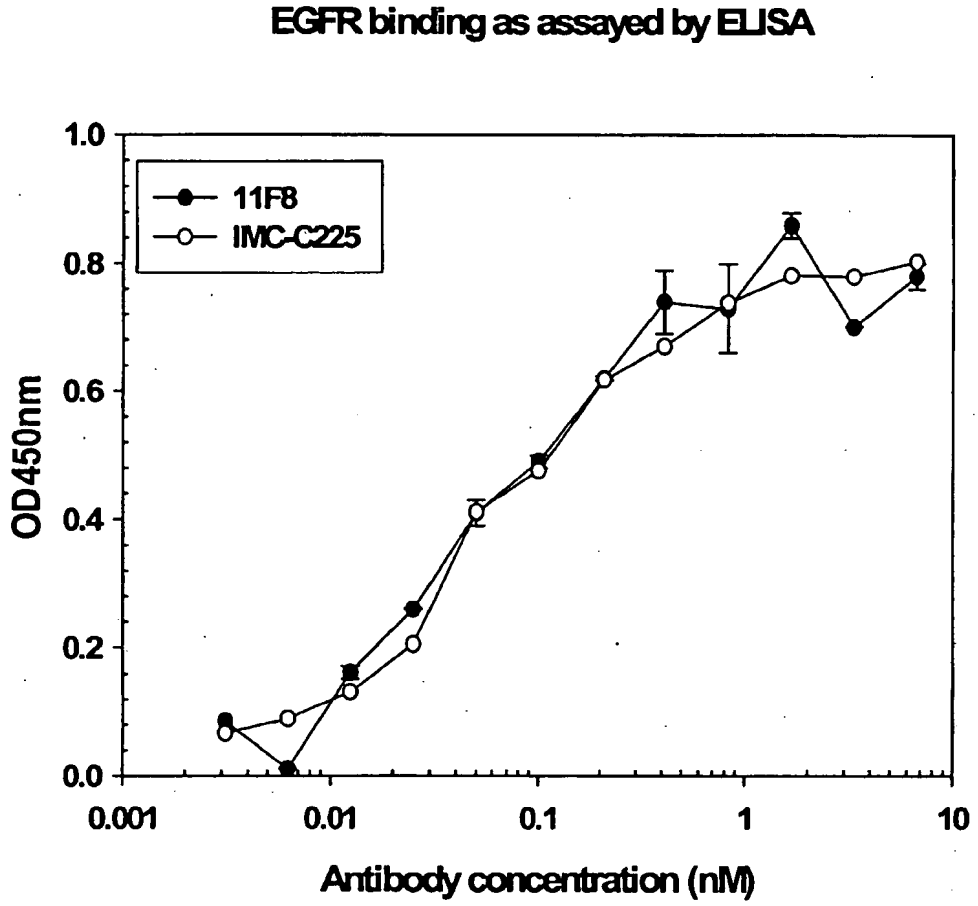

EGFR binding as assayed by ELISA
11F8
IMC-C225
OD450nm
1.0
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
Antibody concentration (nM)

FIG. 2

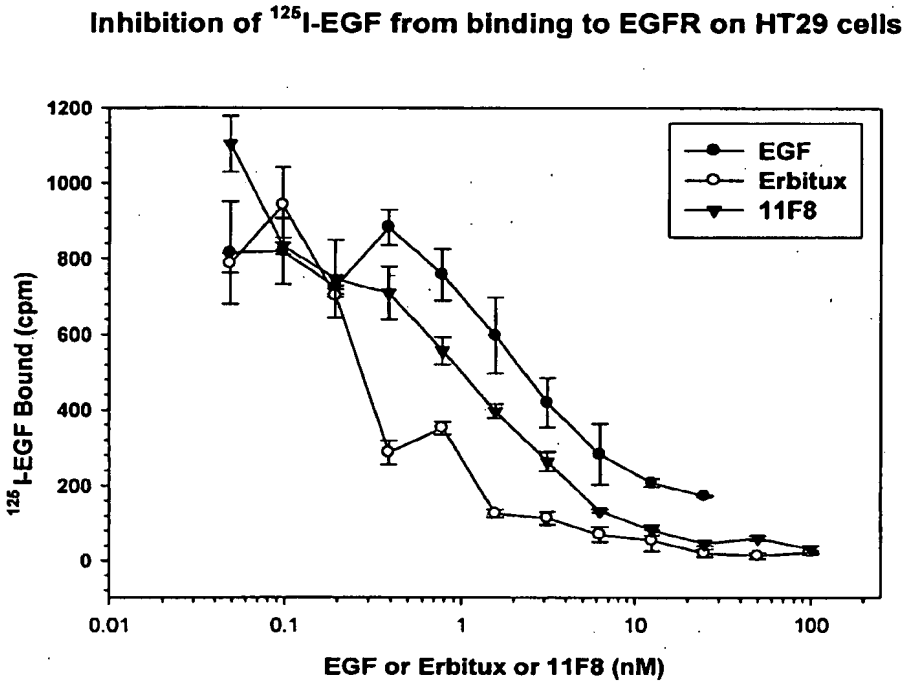

Inhibition of 125I-EGF from binding to EGFR on HT29 cells
125I-EGF Bound (cpm)
1200
1000
800
600
400
200
0
0.01
0.1
1
10
100
EGF or Erbitux or 11F8 (nM)
EGF
Erbitux
11F8

FIG. 3

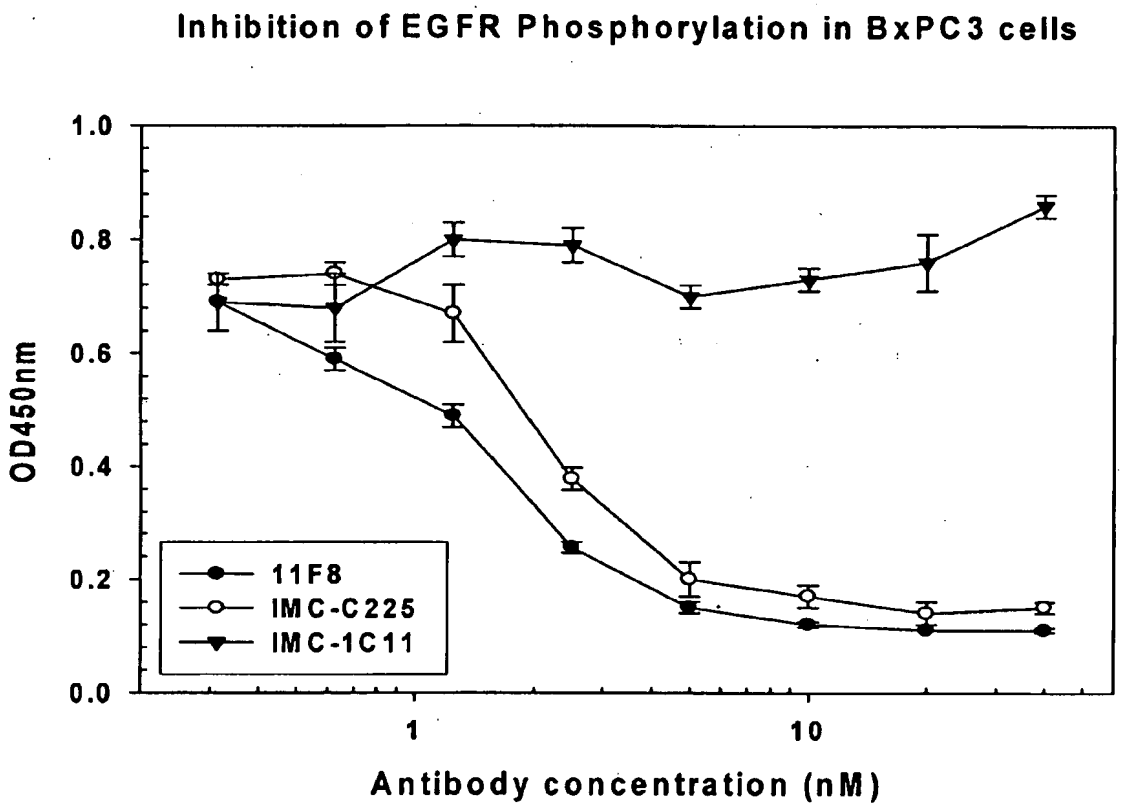

Inhibition of EGFR Phosphorylation in BxPC3 cells
OD450nm
1.0
0.8
0.6
0.4
0.2
0.0
1
10
Antibody concentration (nM)
11F8
IMC-C225
IMC-1C11

FIG. 4

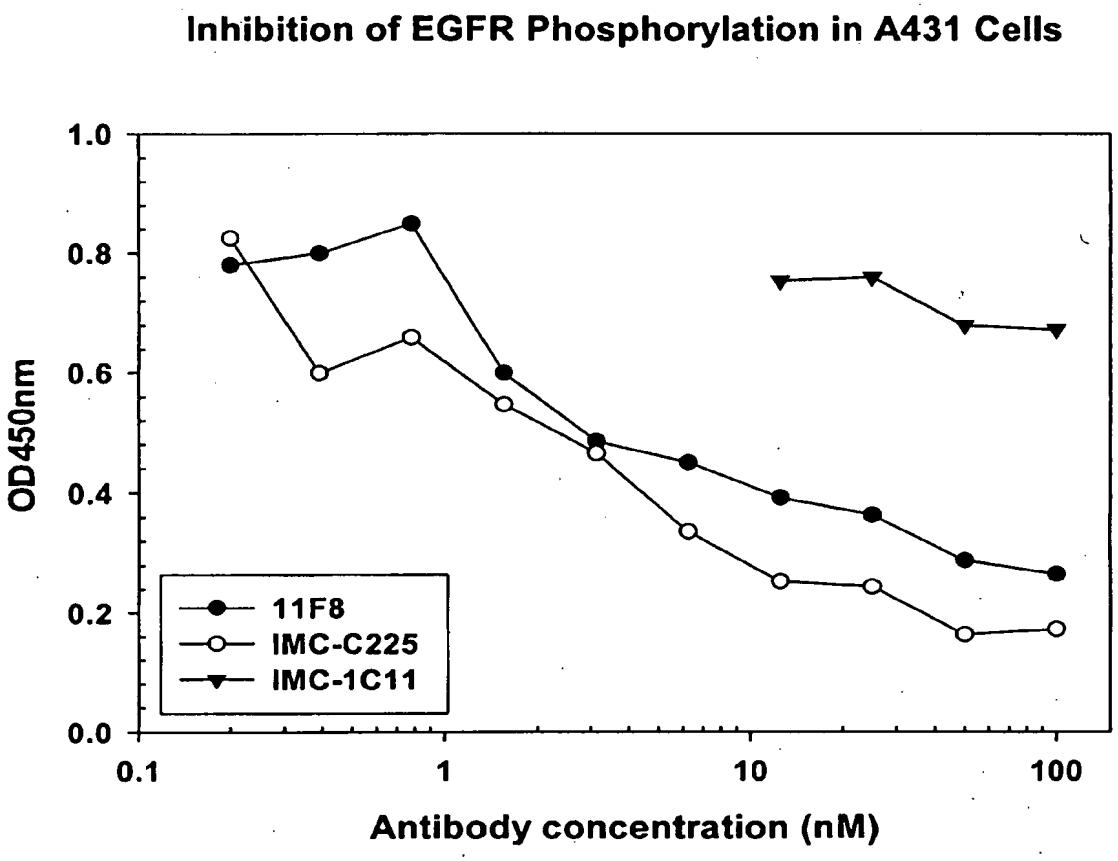

Inhibition of EGFR Phosphorylation in A431 Cells
OD450nm
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
100
Antibody concentration (nM)
11F8
IMC-C225
IMC-1C11

FIG. 5

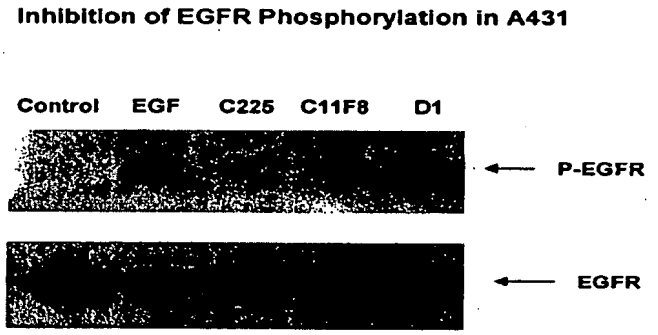


FIG. 5A

FIG. 5B

FIG. 6

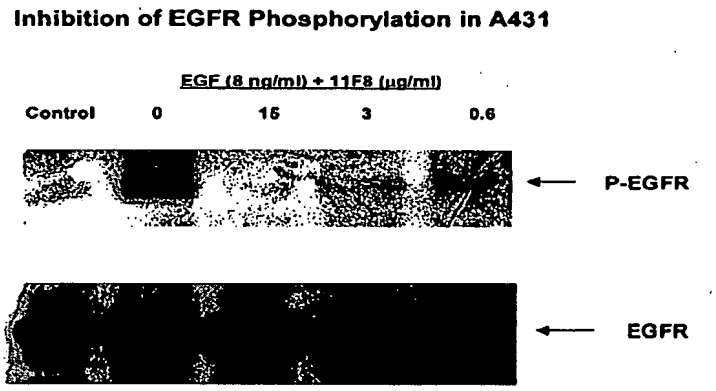


FIG. 6A

FIG. 6B

FIG. 7

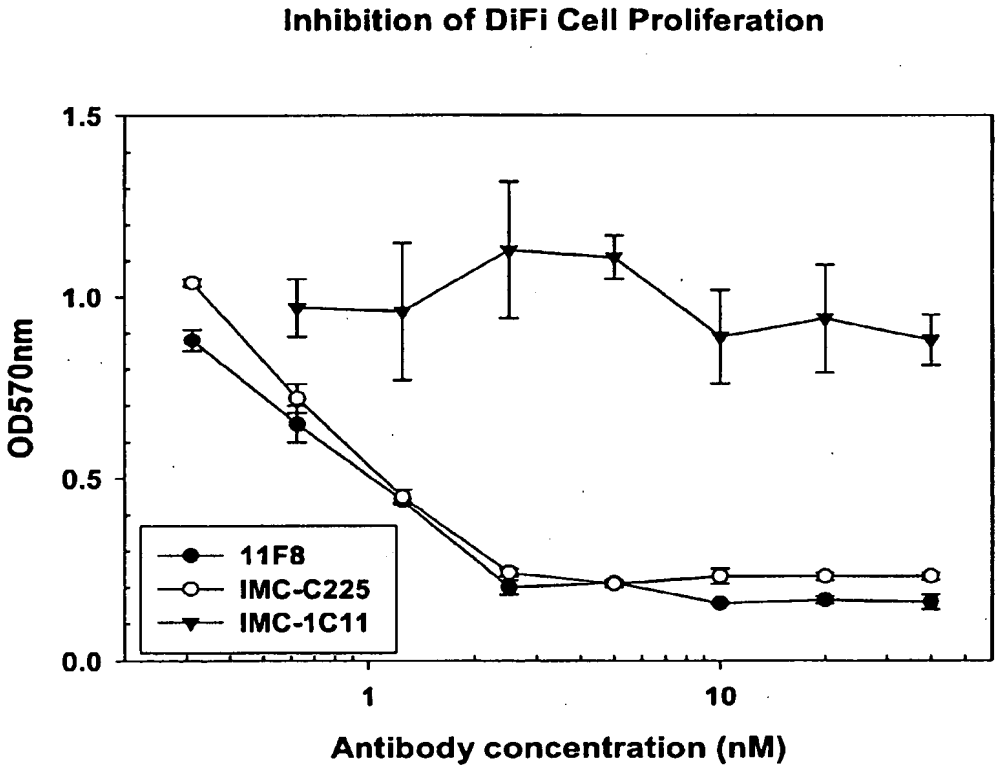

Inhibition of DiFi Cell Proliferation
OD570nm
1.5
1.0
0.5
0.0
1
10
Antibody concentration (nM)
11F8
IMC-C225
IMC-1C11

FIG. 8

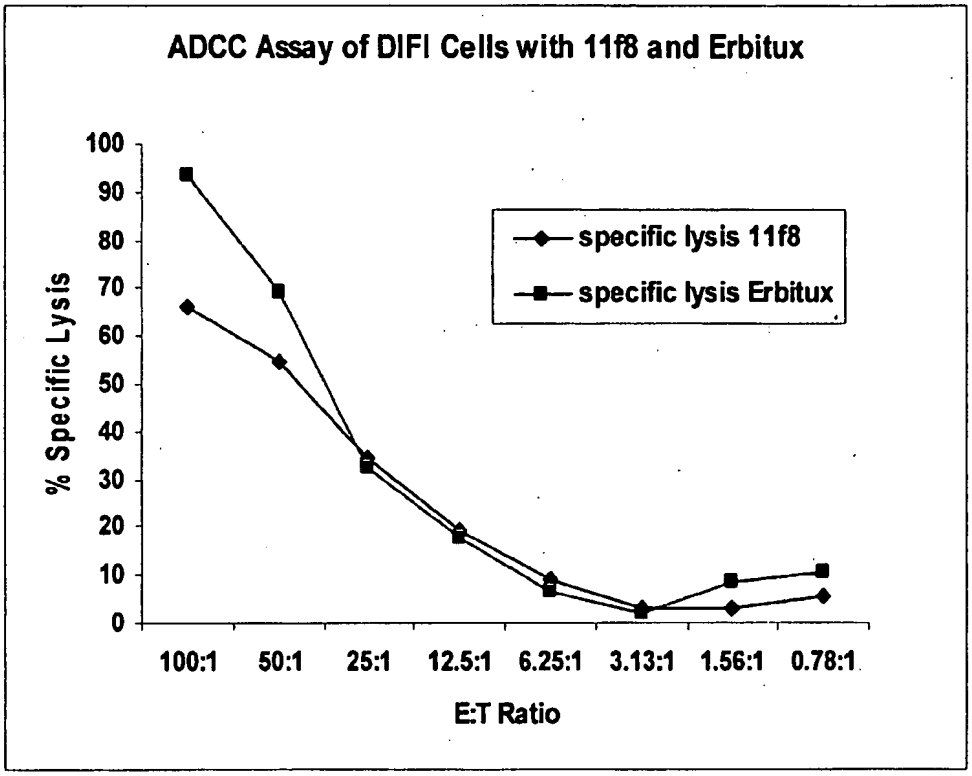

ADCC Assay of DIFI Cells with 11f8 and Erbitux
% Specific Lysis
100
90
80
70
60
50
40
30
20
10
0
specific lysis 11f8
specific lysis Erbitux
100:1
50:1
25:1
12.5:1
6.25:1
3.13:1
1.56:1
0.78:1
E:T Ratio

FIG. 9

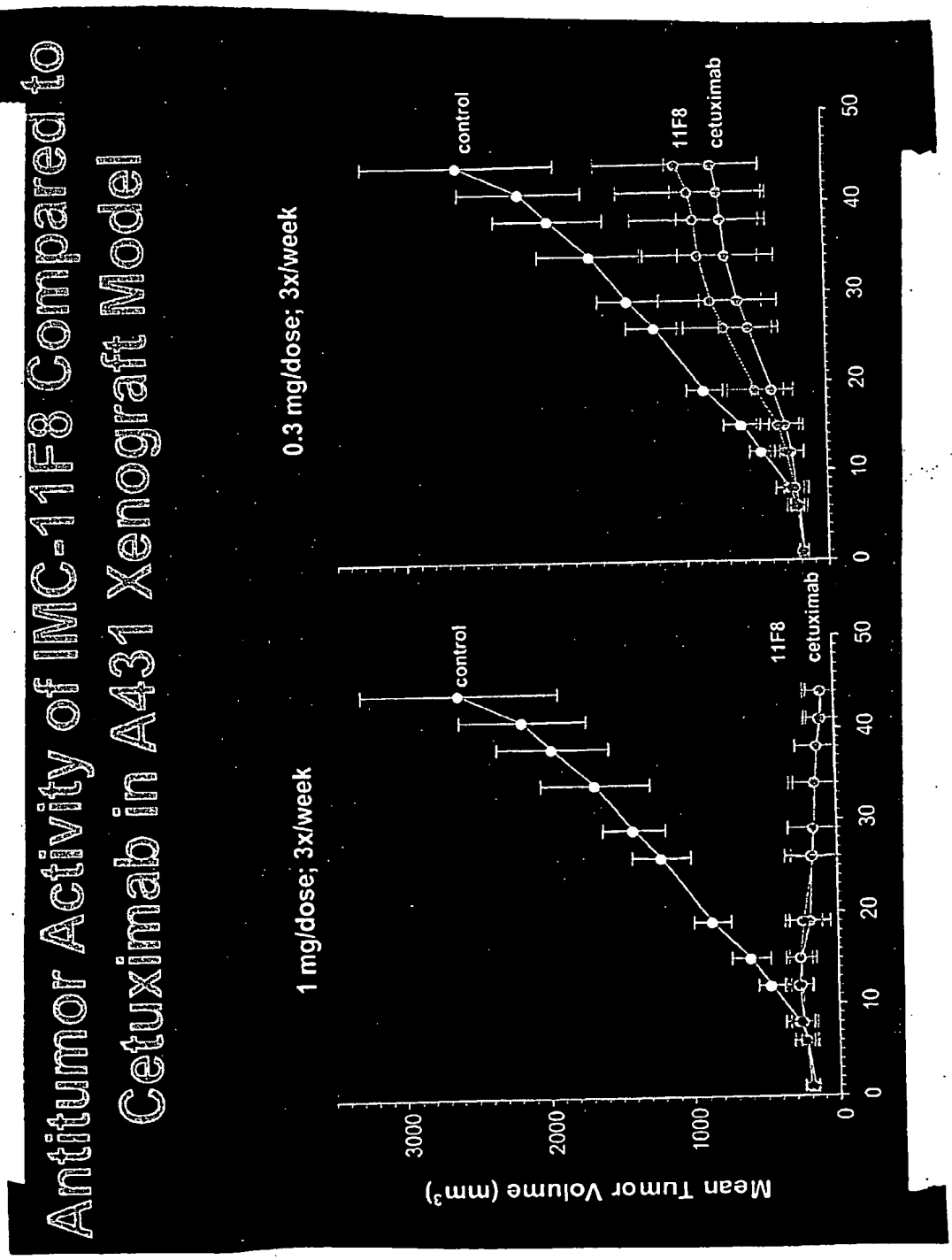
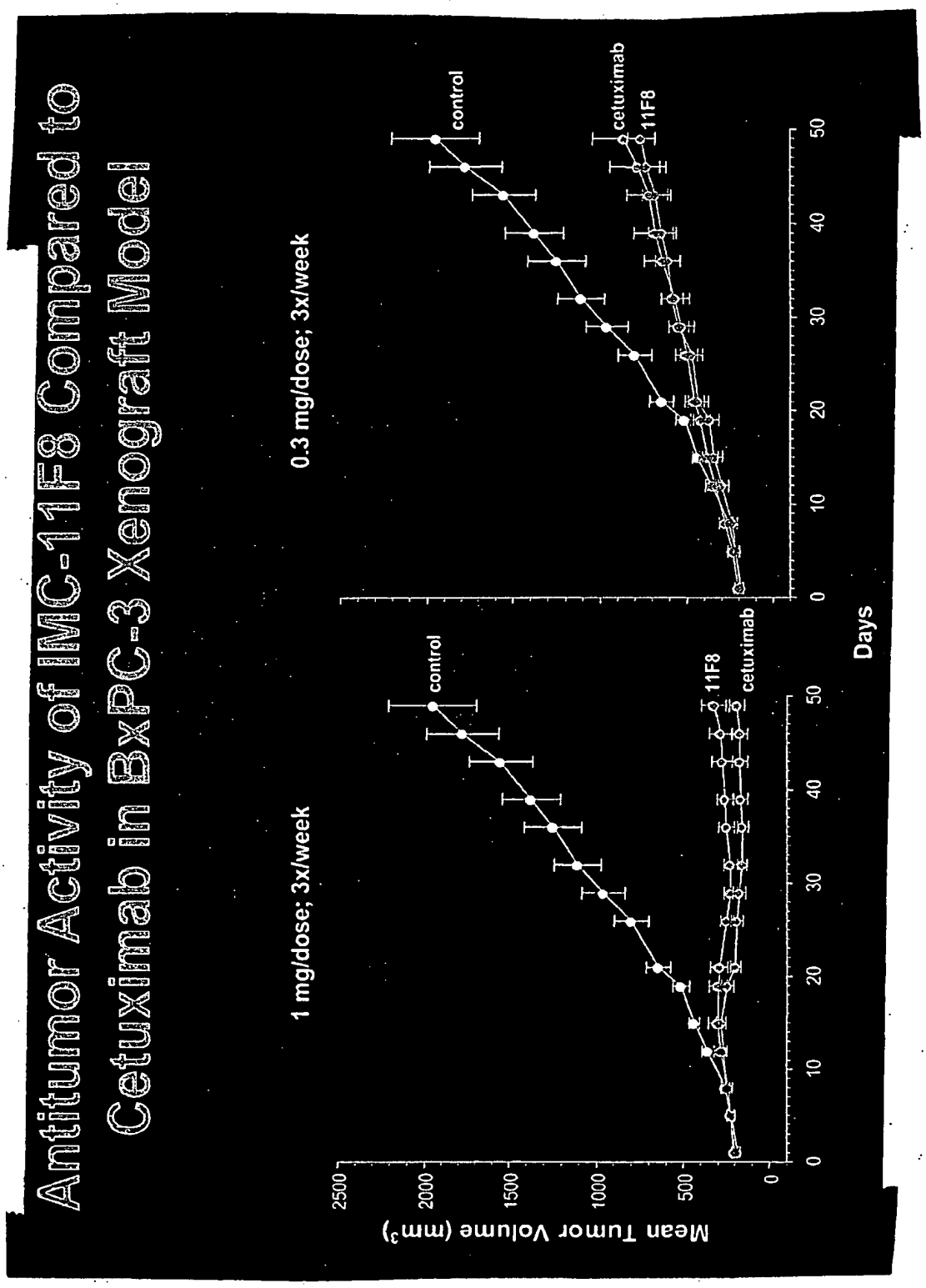

Antitumor Activity of IMC-11F8 Compared to Cetuximab in A431 Xenograft Model
1 mg/dose; 3x/week
0.3 mg/dose; 3x/week
control
11F8
cetuximab
Mean Tumor Volume (mm³)
0
1000
2000
3000
0
10
20
30
40
50

FIG. 10


Antitumor Activity of MC-11F8 Compared to Cetuximab in BxPC-3 Xenograft Model
1 mg/dose; 3x/week
0.3 mg/dose; 3x/week
control
11F8
cetuximab
Mean Tumor Volume (mm³)
Days
0
500
1000
1500
2000
2500
10
20
30
40
50

AMINO ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR1 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

Ser Gly AspTyr Tyr Trp Ser (SEQ ID NO:1)

NUCLEIC ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR1 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

agc agt ggt gat tac tac tgg agt (SEQ ID NO:2)

AMINO ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR2 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

Tyr Ile Tyr Tyr Ser Gly Ser Thr Asp Tyr Asn Pro Ser Leu Lys Ser (SEQ ID NO:3)

NUCLEIC ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR2 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

tac atc tat tac agt ggg agc acc gac tac aac ccg tcc ctc aag agt (SEQ ID NO:4)

AMINO ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR3 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

Val Ser Ile Phe Gly Val Gly Thr Phe Asp Tyr (SEQ ID NO:5)

NUCLEIC ACID SEQUENCE OF HEAVY CHAIN VARIABLE CDR3 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

gtg tcg att ttt gga gtg ggg aca ttt gac tac (SEQ ID NO:6)

HEAVY CHAIN VARIABLE REGION AMINO ACID SEQUENCE, IMC-11F8
(SEQ ID NO:7)

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

Asp Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Val Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Val Ser Ile Phe Gly Val Gly Thr Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

HEAVY CHAIN VARIABLE REGION DNA SEQUENCE, IMC-11F8
(SEQ ID NO:8)

```
cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tca cag    48
acc ctg tcc ctc acc tgc act gtc tct ggt ggc tcc atc agc agt ggt    96
gat tac tac tgg agt tgg atc cgc cag ccc cca ggg aag ggc ctg gag   144
tgg att ggg tac atc tat tac agt ggg agc acc gac tac aac ccg tcc   192
ctc aag agt cga gtc acc atg tcc gta gac acg tcc aag aat cag ttt   240
tcc ctg aag gtc aac tct gtg acc gcc gca gac acg gct gtg tat tac   288
tgt gcg aga gtg tcg att ttt gga gtg ggg aca ttt gac tac tgg ggc   336
cag ggc acc ctg gtc acc gtc tca agc                              363
```

AMINO ACID SEQUENCE OF LIGHT CHAIN VARIABLE CDR1 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala (SEQ ID NO:9)

NUCLEIC ACID SEQUENCE OF LIGHT CHAIN VARIABLE CDR1 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

agg gcc agt cag agt gtt agc agc tac tta (SEQ ID NO:10)

AMINO ACID SEQUENCE OF LIGHT CHAIN VARIABLE CDR2 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

Asp Ala Ser Asn Arg Ala Thr (SEQ ID NO:11)

NUCLEIC ACID SEQUENCE OF LIGHT CHAIN VARIABLE CDR2 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

gat gca tcc aac agg gcc act (SEQ ID NO:12)

AMINO ACID SEQUENCE OF LIGHTCHAIN VARIABLE CDR3 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

His Gln Tyr Gly Ser Thr Pro Leu Thr (SEQ ID NO:13)

NUCLEIC ACID SEQUENCE OF LIGHTCHAIN VARIABLE CDR3 OF HUMAN ANTI-EGFR ANTIBODY, IMC-11F8

cac cag tat ggt agc aca cct ctc (SEQ ID NO:14)

LIGHT CHAIN VARIABLE REGION AMINO ACID SEQUENCE, IMC-11F8
(SEQ ID NO:15)

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys His Gln Tyr Gly Ser Thr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Ala Glu Ile Lys
100 105

LIGHT CHAIN VARIABLE REGION DNA SEQUENCE, IMC-11F8
(SEQ ID NO:16)

```
gaa att gtg atg aca cag tct cca gcc acc ctg tct ttg tct cca ggg    48

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agc agc tac    96

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc   144

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc   192

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct   240

gaa gat ttt gca gtg tat tac tgt cac cag tat ggt agc aca cct ctc   288

act ttc ggc gga ggg acc aag gcg gag atc aaa                      321
```

IMC-11F8 HEAVY CHAIN VARIABLE REGION, IMC-11F8
(SEQ ID NO:17)

Fr1

```
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGAC 50
 Q  V  Q  L  Q  E  S  G  P  G  L  V  K  P  S  Q  T
                                          CDR1
CCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGATT 100
  L  S  L  T  C  T  V  S  G  G  S  I  S  S  G  D
          Fr2
ACTACTGGAGTTGGATCCGCCAGCCCCCAGGGAAGGGCCTGGAGTGGATT 150
Y  Y  W  S  W  I  R  Q  P  P  G  K  G  L  E  W  I
    CDR2
GGGTACATCTATTACAGTGGGAGCACCGACTACAACCCGTCCCTCAAGAG 200
 G  Y  I  Y  Y  S  G  S  T  D  Y  N  P  S  L  K  S
  Fr3
TCGAGTCACCATGTCCGTAGACACGTCCAAGAATCAGTTTCCCTGAAGG 250
  R  V  T  M  S  V  D  T  S  K  N  Q  F  S  L  K
                                              CDR3
TCAACTCTGTGACCGCCGCAGACACGGCTGTGTATTACTGTGCGAGAGTG 300
V  N  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  V
                                    Fr4
TCGATTTTTGGAGTGGGGACATTTGACTACTGGGGCCAGGGCACCCTGGT 350
 S  I  F  G  V  G  T  F  D  Y  W  G  Q  G  T  L  V

CACCGTCTCAAGC 363
  T  V  S  S
```

IMC-11F8 Heavy chain variable region.

IMC-11F8 LIGHT CHAIN VARIABLE REGION, IMC-11F8
(SEQ ID NO:18)

Fr1

```
GAAATTGTGATGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA 50
 E  I  V  M  T  Q  S  P  A  T  L  S  L  S  P  G  E
```

CDR1

```
AAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAG 100
  R  A  T  L  S  C  R  A  S  Q  S  V  S  S  Y  L
```

Fr2 **CDR2**

```
CCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGAT 150
A  W  Y  Q  Q  K  P  G  Q  A  P  R  L  L  I  Y  D
```

Fr3

```
GCATCCAACAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTC 200
 A  S  N  R  A  T  G  I  P  A  R  F  S  G  S  G  S

TGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTG 250
  G  T  D  F  T  L  T  I  S  S  L  E  P  E  D  F
```

CDR3 **Fr4**

```
CAGTGTATTACTGTCACCAGTATGGTAGCACACCTCTCACTTTCGGCGGA 300
A  V  Y  Y  C  H  Q  Y  G  S  T  P  L  T  F  G  G

GGGACCAAGGCGGAGATCAAA 321
 G  T  K  A  E  I  K
```

IMC-11F8 Light chain variable region

LINKER AMINO ACID SEQUENCES

$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ (SEQ ID NO:19).

(Gly-Gly-Gly-Gly-Ser) (SEQ ID NO:20).

$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO:21).